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LARVAL BIOLOGY OF *BRIAROSACCUS CALLOSUS* BOSCHMA (CIRRIPEDIA: RHIZOCEPHALA)

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Abstract.—*Briarosaccus callosus,* a rhizocephalan barnacle hosted by three species of king crabs (*Paralithodes camtschatica, P. platypus, Lithodes aequispina*) in southeastern Alaska, was reared through four naupliar stages and a cyprid stage in the laboratory. The second to fourth naupliar stages have a characteristic dorsal shield and the third and fourth stages have a broader antennule with one less seta than the first and second stages. No other differences were observed in the appendages and setation among the four stages. The cyprid stage developed within 20–29 days at 6 to 8°C. A wide variation in size of cyprids from *P. platypus* and *L. aequispina* hosts may be attributed to sexual dimorphism, with males larger as reported in other Rhizocephala. Based on larval morphology the rhizocephalans hosted by *P. platypus* and *L. aequispina* are considered to be conspecific.

The rhizocephalan *Briarosaccus callosus* Boschma is a widely distributed parasite of many lithodid species (Boschma 1962, 1970; Arnaud and Do-Chi 1977; Somerton 1981; Hawkes et al. 1985). Rhizocephalans are recognizable as Cirripedia only during their larval stages, and the lack of hard parts in adults renders rhizocephalan taxonomy difficult. *Briarosaccus callosus* was assigned to a new genus and species based on the large size of its externa, robust appearance, and cuticular retinacula and thickness of the mantle (Boschma 1930, 1970).

Detailed descriptions of the adult externae of *B. callosus* have been given previously by Boschma (1930, 1962), Boschma and Haynes (1969) and Bower and Sloan (in press). However, the larval stages and the life history of this and many other rhizocephalans have not been described. Boschma (1927) noted that the larval forms of Rhizocephala were of special interest from a taxonomic point of view because of the paucity of useful taxonomic characters in adults. Boschma (1927) reported only a few rhizocephalans in which the larval stages are known, but stated that the different larval morphologies provided sufficient evidence to distinguish species. However, few of Boschma's subsequent taxonomic works included larval descriptions.

Histological examinations of Alaskan king crab parasitized by *B. callosus* have raised dbouts as to whether the parasite is conspecific in all species of lithodid crabs (Sparks and Morado, in press). The barnacle rootlets in parasitized red and golden king crabs, *Paralithodes camtschatica* and *Lithodes aequispina,* are different, perhaps due to the difference in hosts or possibly because they represent two different species of parasites. Blue king crabs, *P. platypus,* have different histological and physiological responses to the parasite than golden king crabs. Golden king crabs also have a much lower rate of multiple infection than blue king crabs (Shirley et al. in press). Whether one or more species of *Briarosaccus* occurs in Alaskan king crabs is potentially important for management strategy of crab populations. Life history information may also help explain why, in southeastern

Alaska, certain populations of *P. platypus* and *L. aequispina* are heavily parasitized while populations of *P. camtschatica,* the red king crab, are not (Hawkes et al. in press).

This report describes the naupliar and cyprid stages of *B. callosus,* other details of the parasite's life history, and addresses the question of conspecificity of specimens from red, blue, and golden king crab hosts.

Materials and Methods

Parasitized *L. aequispina* (n = 19) from Lynn Canal near Haines, Alaska (59°20′N, 135°20′W), *P. platypus* (n = 19) from Glacier Bay (58°50′N, 135°50′W), and *P. camtschatica* (n = 3) from the Juneau area (58°20′N, 134°30′W) were maintained in separate-flowing sea water tanks. Crabs were tagged for individual identification with a numbered plastic disc (fry tags) glued to the middle of each carapace. Crabs were fed mussels and fish diet, ad libitum.

The maturity of *B. callosus* eggs within externae was monitored every 2–3 weeks by microscopic examination. Eggs were extracted from the outer mantle cavity with a Pasteur pipette inserted through the papilla. When ova contained completely formed nauplii, spawning of the externa was imminent. Crab hosts with prespawning externae were placed in a closed vessel of filtered sea water until the natural extrusion of barnacle larvae occurred. After extrusion, fractional aliquots of larvae from an entire brood were counted to provide an estimate of the number of nauplii in a single spawning. Immediately after spawning, each externa became flaccid from the emptying of the outer mantle chamber. By monitoring ovarian maturity in barnacle externae, estimates were made of brood development and periodicity of release.

During March through July, 1984, *Briarosaccus* larvae from all three host species of king crab were reared in 5 liter glass containers and in petri dishes at ambient sea water temperatures. Water temperature increased from 6 to 8°C during that period of time. Like other rhizocephalans, *B. callosus* larvae are nonfeeding (Yanagimachi 1961b; Ritchie and Hoeg 1981). Specimens of reared larvae were periodically preserved in 10% buffered formalin. Drawings were made of larvae with the aid of a camera lucida mounted on a compound microscope. Measurements were made with an ocular micrometer. At least five nauplii of each stage were dissected and examined to determine the setation formula and possible morphological differences among broods from different host species (Bassindale 1936). The lengths of the dorsal shield and the body, measured from the anterior border to the end of the caudal spine, were determined from a minimum of 30 nauplii of each stage. Mean body lengths of cyprids from each brood were determined from a minimum of 17 larvae. Student's t tests were used to compare mean values ($\bar{x}$) which were given ± one standard deviation. Probabilities less than 0.05 are considered significant and those less than 0.01 are considered highly significant.

Results

Larvae of *B. callosus* were released from externae as stage I nauplii. Broods of larvae varied in number from $312 \pm 96 \times 10^3$ to $388 \pm 127 \times 10^3$. The time interval between broods was not significantly different ($P = 0.81$) for *B. callosus*

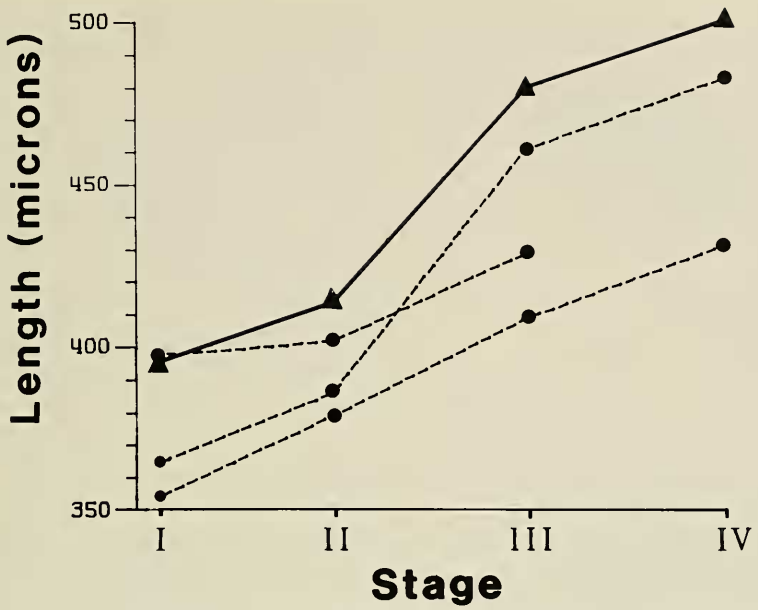

Fig. 1. Mean body lengths of naupliar stage larvae of *Briarosaccus callosus* from broods hosted by one golden (triangles) and three blue king crabs (circles).

infecting blue king crabs (n = 4, 45 ± 5.3 days) and golden king crabs (n = 8, 48 ± 6.2 days) during June to August. Seven different broods were obtained for larval rearing of *B. callosus,* but only three of these were successfully cultured to cyprids. The most successful rearing technique was maintaining small numbers of larvae in petri dishes of sea water.

Briarosaccus callosus has four naupliar stages (Fig. 1) before metamorphosis to a cyprid. The nauplii have three pairs of jointed appendages (Fig. 2) and are similar in shape to other rhizocephalans described by Boschma (1927), Reinhard (1946) and Schram (1972). The appearance of the appendages and the numbers of setae on them did not vary in nauplii from the different crab hosts.

The first stage nauplius (Fig. 3*a*) is triangular, with frontal horns directed outward and slightly posteriorly, and has a setation formula of 0.1.1.2.1; 0.5.–0.3.1.G; 0.2.–0.4.0.G. It is nearly colorless, contains numerous fat droplets, has no eye spot, and is similar to the specimens that Boschma (1927) found within the mantle cavity of preserved externae of *Sacculina carcini, S. exarcuata, S. neglecta,* and *Peltogaster gracilis.*

Metamorphosis to the second naupliar stage is usually complete within 24 hours after extrusion from the externa. This stage is slightly larger (Fig. 1), and the horns are directed anteriorly. The larva has an oval-shaped dorsal shield (Fig. 3*b–c*), which is easily lost in fixed specimens. This complex structure is marked with ribs forming small diamond-shaped patterns similar to markings reported for other rhizocephalan larvae (Reinhard 1946; Schram 1972). The setation formula for stage II nauplii is the same as for stage I.

Stage III nauplii developed in seven to eight days followed by stage IV in eight to 10 days. Though similar to the second stage these last two naupliar stages are

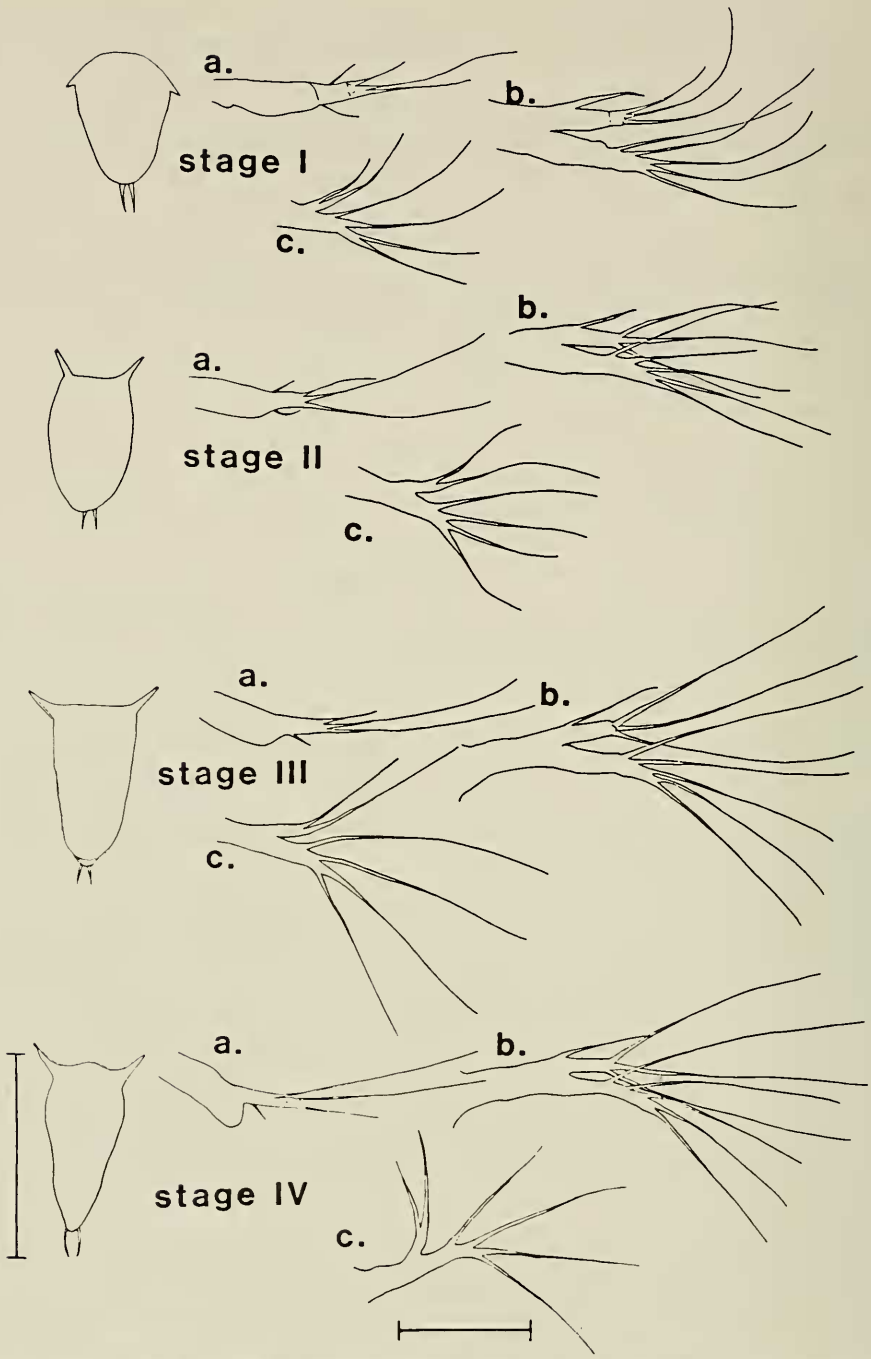

Fig. 2. Body (scale = 500 μm) and appendages (scale = 100 μm) of the 4 naupliar stages of *Briarosaccus callosus*; (a) antennule, (b) antenna, (c) mandible.

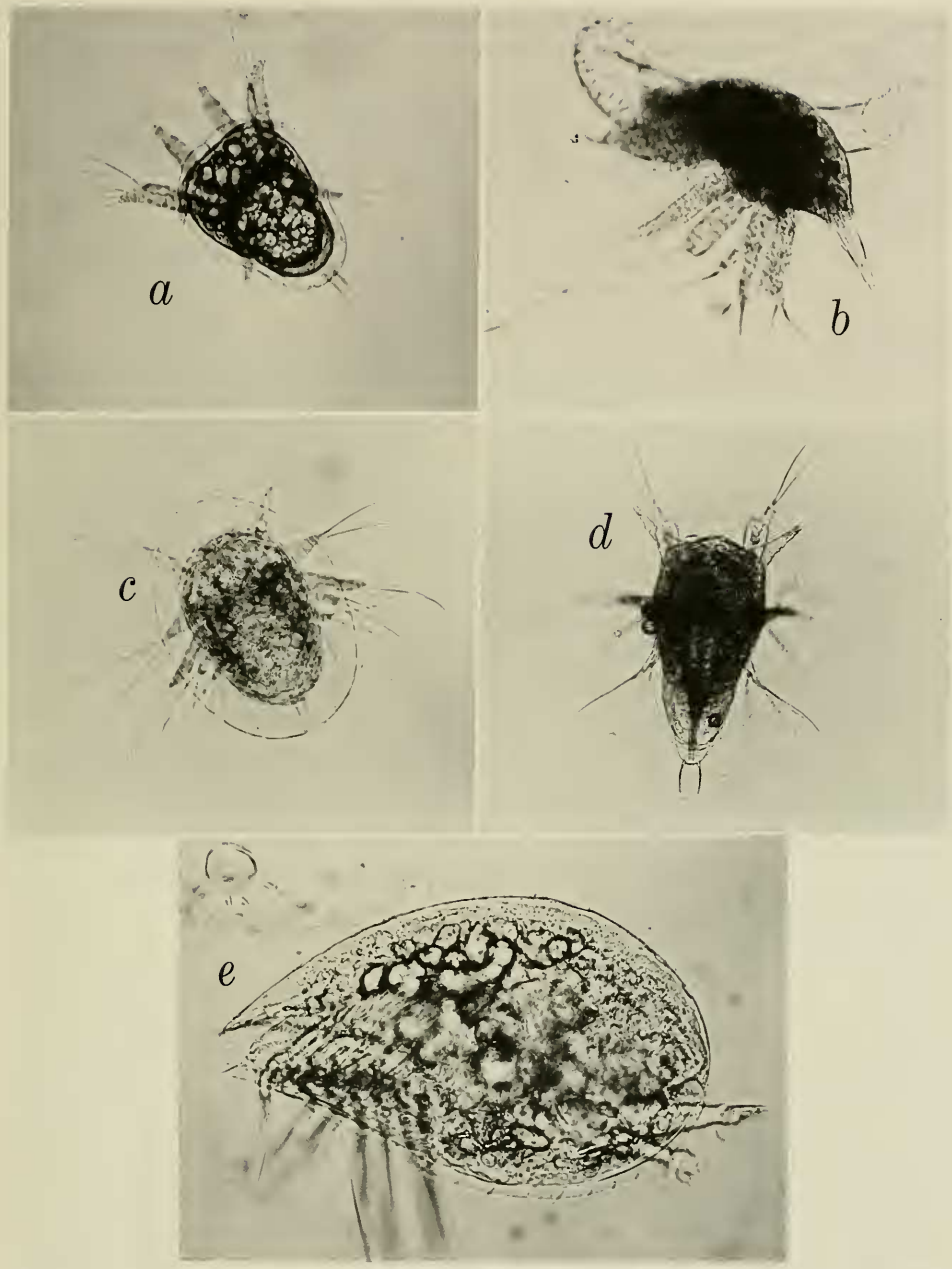

Fig. 3. *Briarosaccus callosus* larvae: *a,* First naupliar stage from a blue king crab host; *b,* Lateral view of second naupliar stage from a golden king crab host; *c,* Ventral view of second naupliar stage from a golden king crab host; *d,* Fourth naupliar stage from a golden king crab host. A developing cyprid is visible in the abdomen; *e,* Cyprid from a blue king crab host.

Table 1.—Naupliar stages of *Briarosaccus callosus*; total lengths for bodies and dorsal shields (carapace) of one brood from a golden king crab and broods from 3 different blue king crabs. Sample size is in parentheses if less than 30.

	Length (μm)	
	Body	Carapace
Golden king crab		
I	393 ± 15.5	
II	413 ± 14.1	546 ± 20.0 (16)
III	478 ± 22.7	657 ± 16.4
IV	500 ± 11.1	615 ± 11.9 (26)
Blue king crab		
I	351 ± 10.5	
II	379 ± 7.7	476 ± 5.41 (24)
III	410 ± 13.0	559 ± 13.1 (9)
IV	437 ± 8.61	538 ± 10.6 (22)
I	362 ± 10.2	
II	386 ± 8.4	504 ± 20.7
III	461 ± 3.05	
IV	483 ± 9.1 (27)	
I	397 ± 16.8 (21)	
II	400 ± 13.9	489 ± 12.5
III	420 ± 14.6	

larger; the antennule is broadened at the base and has one less seta. Consequently, the setation formula for both stages III and IV is 0.1.2.1; 0.5.–0.3.1.G.: 0.2.–0.4.0.G. The dorsal shield becomes slightly smaller between stages III and IV (Table 1). In stage IV the abdomen is more slender and elongate, and the cyprid is visible internally (Fig. 3*d*).

No morphological differences could be found among the larvae of *B. callosus* hosted by the three species of king crab.

Cyprids of *B. callosus* (Fig. 3*e*) were enclosed in a bivalve shell with the antennules projecting beyond the anterior edge. A similar morphology has been reported for other rhizocephalan cyprids (Reinhard 1946; Yanagimachi 1961a). Two of the successful broods were from *P. platypus* hosts (Table 2). Cyprid larvae ($\bar{x}$ = 374 ± 8 μm in length) from one of these broods developed in 29 days at 6 to 7°C while those from the other blue king crab ($\bar{x}$ = 321 ± 12 μm in length) developed in 20 days. This second brood of cyprids remained alive for an additional 16 days at 7 to 8°C. When reared at 4°C, nauplii from this brood only developed to the fourth naupliar stage but survived for 57 days. A third culture of cyprids ($\bar{x}$ = 399 ± 16 μm in length), originating from *L. aequispina,* developed within 25 days at 7–8°C. Cyprid body lengths of all three broods were highly significantly different (Table 2). *Briarosaccus callosus* larvae from a *P. camtschatica* host were successfully reared only to the first naupliar stage ($\bar{x}$ = 387 ± 12.0 μm in length).

Discussion

Very few reports of larval biology of Rhizocephala exist. Newly hatched nauplii were measured by Yanagimachi (1961a) in *Peltogasterella gracilis* and their sex

Table 2.—Measurements of cyprids, parent externae of *Briarosaccus callosus,* and the respective king crab host.

	Host			Parent externa		
King crab host	Length (mm)	Weight (g)	Cyprid length (μm ± SD)	Diameter (mm)	Length (mm)	Weight (g)
Golden	127	1110	399 ± 15	22	48	21.3
Blue	115	1065	374 ± 8	26	44	13.2
Blue	99	556	321 ± 12	38	54	17.8

determined according to size, with males (250–290 μm) being larger than females (207–243 μm). Newly emerged *B. callosus* larvae also varied greatly in size but were larger than larvae of *P. gracilis.* The second to fourth stages of *B. callosus* were similar, having a characteristic dorsal shield similar to *P. paguri* (Reinhard 1946; Schram 1972). However, the stages were not easily differentiated since the size ranges of the body and dorsal shield overlapped slightly. The size difference between male and female was pronounced in all stages. Schram (1970) suggested that the naupliar stages might be recognized by differences in the setation of the appendages. This is certainly true among different species. For example, the setation formulae for the metanauplius of *Peltogaster paguri* (Schram 1972) is different than that for *B. callosus* in the second antenna and mandible. Since setation of the appendages is similar among the naupliar stages of *B. callosus,* it could not be used as a diagnostic character for each instar.

The difference in sizes between the three broods of *B. callosus* cyprid larvae may best be explained by sexual dimorphism (Yanagimachi 1961a; Ritchie and Hoeg 1981; Hoeg 1984) rather than species differentiation, since no morphological distinctions between the nauplii or cyprids were present among broods. Thus, barnacle parasites of blue, golden, and probably red king crabs are considered conspecific. In addition, the periodicity of parasite brood release and seasonality of spawning did not differ between externae on blue or golden king crab hosts, again suggesting that they most likely represent the same species.

The two largest-sized cyprids probably represented male broods while the small-sized brood was probably female. The wide range in sizes may be explained by mixed sex broods as described for *Lernaeodiscus porcellanae* (Ritchie and Hoeg 1981). In *L. porcellanae* a predominance of male cyprids occurred during the winter with females occurring in the summer. Broods of *Sacculina carcini* cyprids have also displayed bimodal size distributions, some broods (<10%) having mostly one sex with a very small proportion of the other sex (Hoeg 1984). Hoeg (1984) concluded that sex determination of *Sacculina carcini* cyprids is difficult for intermediate-sized larvae because of the small size difference between males and females and because the size of both sexes varied between his two sampling periods (May–June and August).

Briarosaccus callosus is the first large cold-water rhizocephalan to have been reared successfully through its free-living larval stages in the laboratory. It is considerably different from other rhizocephalans in its very large externa, larger brood size and larvae, long brood periodicity, and slow larval development rate. *Sylon hippolytes,* a parasite of the prawn, *Spirontocaris lilljeborgi,* in southern Norway, has a much smaller externa with two mantle openings that releases only

one brood averaging 100,000 larvae during its life cycle (Lützen 1981). These larvae are in the cyprid stage at emergence and all are approximately equal in length (170 μm) (Lützen 1981). *Clistosaccus paguri,* a rhizocephalan on the hermit crab, *Pagurus bernhardus,* releases cyprids monthly which are similar to those of *S. hippolytes* in size and appearance (Hoeg 1982). *Peltogasterella gracilis* releases broods of nauplii every two weeks, that become cyprids in about five days at 20°C (Yanagimachi 1961a). These cyprids have longer body lengths and may be sexed according to size (males = 295–336 μm, females = 235–275 μm). This is also true for *L. porcellanae* from California which every 10–14 days releases a brood of a few hundred to approximately 20,000 nauplii which complete development to cyprids in three to five days (Ritchie and Hoeg 1981). Cyprids of *Sacculina carcini* are also similar in size (239–268 μm) and life history (Hoeg 1984). *Peltogaster paguri* produces broods every 30 to 40 days that range in number from 9,800 to 28,000 nauplii (Reinhard 1942b).

The longer larval devleopment in *B. callosus* has implications for its life history. A longer planktonic existence decreases chances for larval survival. The larger brood partly compensates for this and also affords a greater potential for dispersal of larvae to new areas. The latter point may help explain the widespread distribution of this parasite (Boschma 1962).

Although the complete life history of *B. callosus* is still not known, analogies with other rhizocephalans are useful. Ritchie and Hoeg (1981) determined that the smaller female *L. porcellanae* cyprids entered the branchial chambers of the crab host and attached to a gill lamella where metamorphosis from cyprid to kentrogon began immediately. The kentrogon developed a hollow stylet on its ventral side which punctured the host tissues and injected a small mass of cells. These cells proliferated, becoming the female interna and its external reproductive system, the externa. Newly emerged virginal externae do not reach sexual maturity unless hyperparasitized or fertilized by the larger male cyprids (Reinhard 1942a; Yanagimachi 1961a; Ritchie and Hoeg 1981; Hoeg 1984). Yanagimachi (1961a) demonstrated that the sex of larvae is genetically determined in *Peltogasterella gracilis,* since eggs as well as newly hatched nauplii occurred in two sizes which differed in their sex chromosomes. He also provided evidence of the settling sites for large and small cyprids. It is very probable that *B. callosus* has the same reproductive pattern. Two blue king crabs which had no external indication of parasitism were discovered to be parasitized only after necropsy. The rudimentary development of the interna near the gills supports the contention that the gill is the site of invasion. A virginal externa (11 mm total length) on a *P. platypus* did not increase in size over a 5 month period. The larvae of *B. callosus* were also of different sizes, indicating sexual dimorphism. The life history of *B. callosus* is still speculative until infection experiments can be conducted with host crabs.

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